REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

In the April 30, 2004 Office Action, the Examiner has withdrawn all of the previous art rejections. However, the Examiner has made two new rejections citing two new references, and we offer the following comments to address these.

Claims 9-11 and 18-35 are now rejected under 35 U.S.C. §102(b) as anticipated by Hong et al. (U.S. Patent 5,834,253) ("the '253 patent"). The Examiner on pages 2-3 of the Office Action states:

> With reference to instant claims 9-11, 22-23 , 30-31, Hong et al. teach a method for extending an oligonucleotide primer annealed to a DNA template (double-stranded or single-stranded DNA) for direct cycle sequencing (classic Sanger one-step reaction) at temperatures between 45C and 65C and a melting temperature below about 80C (see column 5, lines 1-17, column 12, lines 1-67, column 13, lines 1-7, column 18, lines 60-67, column 19, lines 1-49, column 20, lines 1-21) comprising (i) mixing a template with a primer (sequence primer), four standard ddNTP terminators or their analogs, a DNA polymerase which has proof-reading 3'-5' exonuclease activity, such that the DNA polymerase functions to excise mismatched nucleotides from 3'-terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template under conditions that DNA polymerase repeatedly extends the primer (see column 5, lines 2-12; (ii) effecting cycle primer extension reaction at temperature below 80C (see column 5, lines 13, column 12, lines 8, 50-55, column 19, lines 3-5).

The invention we are claiming relates to methods of using oligonucleotides as DNA primers in amounts exceeding the DNA templates, to generate numerous copies of the templates when the temperatures of the DNA polymerase reaction mixture are subjected to **multiple rounds of cycling fluctuations below 80°C**. The newly extended DNA fragments far exceed the original templates in copy number. This is the principle of the DNA cycle primer extension and cycle sequencing technology.

In the '253 patent, Hong teaches using a *Bacillus stearothermophilus* DNA polymerase with proof-reading 3'-5' exonuclease activity to perform a **single** enzymatic

DNA primer extension for the purpose of ".....sequencing of a DNA strand from a template....". In this patent, there is no description or even suggestion of cycling fluctuations of temperature in the enzymatic reaction mixture for repeated cycle extension of DNA in the practice. For someone of ordinary skill in this art following the teaching of the '253 patent, the newly extended DNA fragments generated in the test tube would not and could not exceed the templates in copy number at the end of the reaction, because the DNA templates cannot be used more than once.

By contrast, our claims 9-11 are concerned with "a method for extending the molecules of a primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction. . . . .(lines 1-4), where step (ii) requires "effecting cycle primer extension reaction(s) at a temperature below about 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs and thereby produce a sequence-specific amplification product." The '253 patent can only be understood to teach single enzymatic DNA primer extension. Specifically, at column 5, lines 1-17 describe "hybridizing a primer to a DNA template to be sequenced". Column 12, lines 1-67 describes "...sequencing a DNA strand, ... hybridizing a primer to a DNA template to be sequenced". Column 12, line 67 to column 13 lines 1-7 teaches "..double-stranded DNA sequencing...." Column 18, lines 60-67 teaches "..standard Sanger protocol..." Column 19, lines 1-49 teaches "...water bath for 2 minutes (elongation/termination reaction)...." Column 20, lines 1-20 teaches "..classic Sanger one-step reaction....". As someone having ordinary skill in this art would appreciate, these statements are concerned with a single enzymatic extension of a primer with a special DNA polymerase, Bst. We submit that the Examiner is mistaken in her statement that the '253 patent teaches "...that DNA polymerase repeatedly extends the primer (see column 5, lines 2-12)" in the Office Action, page 2, last sentence, and that the '253 patent describes the step of "effecting cycle primer extension reaction" in the Office Action at page 3, line 1. The '253 patent does not teach repeated or cycle extension. The words **"cycle primer extension"** do not appear in the '253 patent at all.

With regard to claims 9-11, 22-23, 28, and 30-31, the Examiner cited the '253 patent at column 5, lines 14-17, column 12, lines 14-24, column 12, lines 55-55, column 20, lines 4-12, column 13, lines 1-7 and column 12, lines 56-64, as the basis for rejection. Here, again, none of these citations teach the use of Bst DNA polymerase for cycle primer extension of templates. With regard to claims 9-10, 22, 28, and 30, the Examiner cited the use of glycerol in the Hong patent (column 19, line 6-11) as the basis for rejection. However, the '253 patent teaches the use of 50% glycerol as a preservative for the Bst DNA polymerase. In the instant claims, 10-20% glycerol is used as a melting agent to cause denaturation of the double-stranded DNA for repeated cycle primer extension.

Lastly, with regard to claims 18-21, 24-27 and 32-35, the Examiner noted certain homology between the DNA polymerase sequences of the '253 patent and SEQ ID Nos 1-4. Even assuming arguendo that this is the case, it remains that all of these method claims also require enzymatic cycle primer extension reaction—which is not taught by the '253 patent at all.

In summary, the '253 patent cannot anticipate any of our claims 9-11 and 18-35, as this patent only discloses a single DNA primer extension—there is no suggestion whatsoever of any repeated or cycle primer extension, which is required in all of our claims.

Claims 1-8 are rejected under 35 U.S.C. §103(a) as obvious over Walker (U.S. Patent 5,7612,124) in view of Hong et al. (U.S. Patent No. 5,834,253) ("the '253 patent").

The Walker patent teaches amplification of nucleic acids at a single temperature **(without cycling)** employing a DNA polymerase in conjunction with an endonuclease. In this technology, it allows the endonuclease to nick the polymerized DNA strand such that the polymerase will displace a DNA strand without digestion while generating a newly polymerized strand. It deals with a technology referred to as "Strand Displacement Amplification," as the title indicates. All the specific lines and paragraphs cited by the Examiner from this Walker patent are descriptions of various DNA polymerases used at

different temperature for a single temperature primer extension associated with strand displacement. There is neither description nor allusion to cycle extension.

The irrelevance of the '253 patent is explained above, and applies equally here. Given the serious deficiencies in each of the '253 patent and Walker, we submit that it is unreasonable to assume that a fair reading of both these references would have lead someone having ordinary skill in this art to our invention. Someone having ordinary skill in this art would not have been able to combine the single-extension methods of these two patent to design our claimed methods using repeated low temperature cycle extension of DNA for amplification. It simply would not have been obvious to combine these references to achieve our claimed invention.

Withdrawal of this rejection is therefore respectfully requested.

In summary, then, someone having ordinary skill in this art, and having in hand either or both of the '253 patent or the Walker patent would not have reasonably found our invention described therein.

All of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited.

If the Examiner has any questions or would like to make suggestions as to claim language, the Examiner is encouraged to contact Marlana K. Titus at (301) 977-7227. **[Please note that this is a new telephone number.]**

By: _____
Marlana Titus, Reg. No. 35,843

Nash & Titus, LLC
6005 Riggs Road
Laytonsville, MD 20882
(301) 977-7227